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Development of a phage displayed disulfide-stabilized Fv fragment vaccine against *Vibrio anguillarum*

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Abstract

Anti-idiotype monoclonal antibody 1E10 can mimic the protective epitope of *Vibrio anguillarum* and be used as vaccine to prevent fish infection of *V. anguillarum*. In this study, the variable heavy (V_H) domain and variable light (V_L) domain of mAb1E10 were cloned by RT-PCR and were linked to each other by a disulfide bond engineered at position 44 of V_H and position 105 of V_L that lie between structurally conserved framework positions. Mutated V_H 44 and V_L 105 were inserted into phagemid pCANTAB5E. When co-transfected by recombinant pCANTAB5E and helper phage M13KO7, the host *Escherichia coli* cells secreted disulfide-stabilized Fv fragment (dsFv) which displayed on the surface of filamentous phage. The binding specificity of the phage-displayed dsFv was proved by ELISA method. Protection experiment showed that Japanese flounders can develop high titer of antibody against the dsFv and survival ratio of vaccinated group was significantly different from control groups. Thus, this phage-displayed dsFv may be used as vaccine against *V. anguillarum* in fishery.

Keywords: *Vibrio anguillarum*; Phage displayed disulfide-stabilized Fv fragment; Vaccine; Japanese flounder

1. Introduction

Vibrio anguillarum is one of the species causing the severe diseases in marine culture and feral fishes worldwide. It is a potential pathogen of many fish, such as Japanese flounder [1], Atlantic halibut [2], Rainbow trout [3], etc. [4,5]. Loss of coordination, haemorrhage at the fin base and splenomegaly were frequent findings. It can cause death if left untreated.

Anti-idiotype antibodies bearing an internal image of an epitope have been successfully used as surrogate antigens to induce protective immunity in animals [6–10]. We previously reported the preparation and characterization of monoclonal anti-idiotype antibodies to *V. anguillarum*. It posses the internal image of the protective epitope of *V. anguillarum* and was used as vaccine to prevent infection of *V. anguillarum* [1]. However, production of monoclonal antibodies is costly and fussy by reproducing hybridoma cells. Gene engineering antibody technology can be used to overcome its disadvantages. Fv fragments are the smallest functional modules of antibodies required for high-affinity binding of antigen. The heterodimers of whole IgG or Fab fragments are connected by a disulfide bond. However, Fv fragments are not connected by a disulfide bond, thus, by themselves are unstable [11]. This instability could be overcome by making recombinant Fv molecular in which the V_H and V_L domains are connected by a peptide link, such that the antigen combining site is regenerated in a single protein. However, compared to whole antibodies or Fabs, scFvs have some potential disadvantages. Many scFvs are unstable [12,13] and some have a lower affinity than whole antibodies or Fabs because the peptide linker interferes with binding or does not sufficiently stabilize the V_H and V_L heterodimer [14,15]. An alternative approach for stabilizing Fv fragments is to connect the V_H and V_L domains by a disulfide bond connecting the frameworks of V_H and V_L in a manner that does not interfere with antigen binding. Such disulfide-stabilized Fvs (dsFvs), constructed by introduction of cysteines at defined position in V_H and V_L,

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have improved stability and increased affinity [16–21]. In this paper, we reported the production and characterization of phage-displayed dsFv of monoclonal anti-idiotype antibody to *V. anguillarum*. This phage displayed dsFv can be used as vaccine instead of mAb1E10 to prevent Japanese flounders from infection of *V. anguillarum*.

2. Materials and methods

2.1. Cloning of V_H and V_L

Polyadenylated mRNA was isolated using TRIZOL™ Reagents (Gibco BRL, USA) from 10^7/ml hybridoma cells IE10 which secreted anti-idiotype monoclonal antibody to *V. anguillarum* [1]. cDNA was produced from the mRNA using Superscript reverse transcriptase kit (Invitrogen, USA) and oligo dT primer.V_H gene and V_L gene of mAb IE10 was amplified using the following primers:

V_H back: 5′ AGGTCCARCTKCTCGAGTCWG 3′
V_H for: 5′ GAC HCATGG GGS TGT YGT GCT AGC TGM RGA GAC DGT GA 3′
V_L back: 5′ ACG TTT CAG CTC CAG CTT GG 3′
V_L for: 5′ GAT RTT KTG ATG ACC CAR AC 3′
(K = G or T, W = T or A, H = A, C or T, S = C or G, Y = T or C, M = A or C, R = A or G, D = A, G or T)

The PCR reaction for V_H using following thermo cycle parameters: 25 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, followed a final extension at 72 °C for 7 min. The following thermo cycle reaction parameters were used for V_L gene PCR: 25 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min, followed by a final extension at 72 °C for 7 min.

Gel-extracted V_H gene and V_L gene PCR products were separately cloned into the PMD 18-T vector (Takara Biotechnology Co. Ltd, China) and transfected into *E. coli* $DH_{5\alpha}$ for propagating. Plasmid DNA was then purified and digested by restriction enzymes *Eco*RI and *Hin*dIII for identification of clones with proper insertion. The nucleotide sequence of plasmids which contained V_H or V_L insert were confirmed by DNA sequencing. V_H sequence and V_L sequence were analyzed, according to the Kabat [22].

2.2. Site-directed mutagenesis of V_H and V_L [23,24]

V_H gene mutation: Firstly, two PCR reactions were done individually. Reaction 1 used V_H back and mutagenesis oligonucleotides 5′ CAGTGTCTGGAATGGATT GGATAC 3′ as PCR primers. Reaction 2 used V_H for and mutagenesis oligonucleotides 5′ TTCCAGACACTGTCCAGGC-CTCTGTTTTA 3′ as PCR primers. Both of the reactions used the same V_H cDNA cloning plasmid as template. The PCR products of the two reactions were gel-extracted separately and mixed as the template for the third PCR reaction proceeding PCR overlap extension by using V_H back and V_H for as PCR primers. The following thermocycling reaction parameters were used for the third PCR reaction: denaturation at 94 °C for 5 min followed by 7 cycles of 40 s at 94 °C, 40 s at 54 °C, 1 min at 72 °C, and finally a 3 min extension at 72 °C without primers. After adding the primers V_H back and V_H for, we repeated the denaturation at 94 °C for 5 min followed by 25 cycles of 30 s at 94 °C, 30 s at 54 °C, 30 s at 72 °C, and a final extension at 72 °C for 7 min. The mutated V_H gene was cloned into the PMD 18-T vector for identification by sequencing. V_L gene mutation: using V_L for and mutagenesis oligonucleotides 5′ ACGTTT CAG CTC CAG CTT GGT CCC ACA ACC GAA CGT-3′ as primers and V_L gene cDNA cloning plasmid as template for PCR. The mutated V_L gene was then cloned into the PMD 18-T vector for identification by sequencing.

2.3. Construction of recombinant dsFv phagemid

Overlap-extension-PCR method was used, as described previously [24,25]. The *Sfi* I site at the 5′ end of mutated V_H gene, ribosome attachment site (SD) and part of the pelB signal sequence at the 3′ end of mutated V_H gene were introduced by two consecutive PCR reactions. The first PCR primers were PH5: 5′ CATGCCATGACTCGC-GGCCCAGCCGGCCTCTGGGGCTGAACTGGCAAAA-CCT 3′ (the underlined sequence was *Sfi* I sequence) and PH3: 5′ GCAGGTATTTCATATTTCTCTCCTTGG-TTAAGCTGAGGAGACGGTGAGAGTGGT 3′ (the underlined sequence were Stop, SD, start and part of pelB signal sequence). The PCR product was purified and used as template for the second PCR. The second PCR primers were PH5 and PH3ext: 5′ AGCAGCAGACCA GCAGCAGCGGTCGGCAGCAGGTATTTCATATTTCT-CTCCTTGG 3′ (the underlined sequence was complementary to PH3, the non-underlined sequence was another part of pelB signal sequence). The PCR product was named V_{Hext}.

The *Not* I site at the 3′ end of mutated V_L gene and the rest of pelB signal sequence at the 5′ end of mutated V_L gene were introduced by two consecutive PCR reactions. The first PCR primers were PL5: 5′ CCTCGCTGCCCAGC-CGGCGATGGCCGAGATGTTGTGATGACCCAGACT 3′ (the underlined sequence will be complementary to PL5ext) and PL3: 5′ AACAGTTTCTGCGGCCGCCTTGGT CCCACAACCGAACGT 3′ (the underlined sequence was *Not* I sequence).The PCR product was purified and used as template for the second PCR. The second PCR primers were PL3 and PL5ext: 5′ TGCCGACCGCTG-CTGCTGGTCTGCTGCTCCTCGCTGCCCAGCCGGCG-ATGGCCGA 3′ (the non-underlined sequence was complementary to PH3ext, the underlined sequence was complementary to PL5, both of them were pelBss sequence). The PCR product was named V_L ext.

Finally, the V_H ext and V_L ext were assembled by PCR overlap-extension method. The PCR primers were PH5 and PL3. The following thermo cycle reaction parameters were